

What is claimed is:

1. An assay for determining the activity of a TPP utilizing enzyme comprising the step of monitoring production of NADH by fluorescence.
2. The assay of embodiment 1, wherein the TPP utilizing enzyme is transketolase, alpha-ketoglutarate dehydrogenase or pyruvate dehydrogenase.
5
3. The assay of embodiment 1, wherein the activity is monitored by GAPDH-catalyzed conversion of NAD to NADH, resulting in the production of fluorescent NADH,
4. The assay of embodiment 1, wherein the activity is measured from homogenized cell samples without isolating cell components.
10
5. The assay of embodiment 4, wherein the samples are tumors, blood and tissues, fresh or frozen, without further purification.
6. An assay for determining the activity an enzyme in an oxidative ribose-5-phosphate generating pathway, comprising the step of monitoring production of NADPH by fluorescence.
15
7. The assay of embodiment 6, wherein the enzyme is glucose-6-phosphate dehydrogenase.
8. The assay of embodiment 6, wherein the activity is monitored by G6PDH-catalyzed conversion of NADP to NADPH, resulting in the production of fluorescent NADPH,
20
9. The assay of embodiment 6, wherein activity is measured from homogenized cell samples without isolating cell components.
10. The assay of embodiment 9, wherein the samples are tumor cells selected from the group of transformed cell lines, fresh or frozen tumor cells or tissues.
25

11. The assay of embodiment 1 or 6, performed on human tumor cells.
12. The assay of embodiment 1 or 6, performed in multi-well dishes.
- 5 13. The assay of embodiment 1 or 6, wherein the assay is cell-based.
14. The assay of embodiment 1 or 6, wherein fluorescence is measured with excitation at about 340 ± 30 nm and emission measured at about 460 ± 30 nm in kinetic mode.
- 10 15. The assay of embodiment 1 or 6, wherein the amount of total protein per assay is less than about 80 micrograms.
16. A method for monitoring the effectiveness of a TPP mimetic drug treatment in a cancer patient, comprising the step of measuring the activity of a TPP utilizing enzyme by monitoring the production of NADH by fluorescence.
- 15 17. The method according to embodiment 16, wherein the activity is measured by sampling a tumor biopsy or whole blood of the patient before, during or after said drug treatment.
18. The method according to embodiment 16, wherein the measurement of said activity can be used to modify said drug treatment in the patient.
- 20 19. The method according to embodiment 16, wherein the drug treatment is optimized through recurring measurements of said activity in a patient.
- 25 20. The method according to embodiment 16, wherein the TPP utilizing enzyme is transketolase, alpha-ketoglutarate dehydrogenase or pyruvate dehydrogenase.

21. The method according to embodiment 16, wherein the activity is monitored by GAPDH-catalyzed conversion of NAD to NADH, resulting in the production of fluorescent NADH,

22. The method according to embodiment 16, wherein fluorescence is measured with excitation at about 340 ± 30 nm and emission measured at about 460 ± 30 nm in kinetic mode.

5

23. A method for identifying a TPP mimetic drug for use as a therapeutic agent comprising the step of comparing the inhibition by N3PT of a TPP utilizing enzyme with the inhibition by a test TPP mimetic drug.

10

24. The method according to embodiment 23, wherein the inhibition of the TPP utilizing is determined by monitoring the production of NADH by fluorescence.

15

25. The method according to embodiment 24, wherein the activity is monitored by GAPDH-catalyzed conversion of NAD to NADH, resulting in the production of fluorescent NADH.

26. The method according to embodiment 23, wherein the inhibition of the TPP utilizing enzyme is measured by competitive binding in the presence of N3PT or thiamine.

27. The method according to any one of embodiments 23-26, wherein the TPP utilizing enzyme activity is transketolase.

20